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Development and validation of a stable-isotope dilution liquid chromatography–tandem mass spectrometry method for the determination of bisphenols in ready-made meals☆

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ABSTRACT

Due to their growing consumption, ready-made meals are a major dietary component for many people in today's society, representing an important potential route of human exposure to several food contaminants. The recent restrictions in the use of bisphenol A have led the plastic industry to look for alternative chemicals, most of them belonging to the same family of *p,p′*-bisphenols.

The aim of the current work was to develop and validate a method based on stable-isotope dilution liquid chromatography–tandem mass spectrometry for the analysis of bisphenol A and its main analogs – bisphenol S, 4,4′-sulfonylbis(2-methylphenol), bisphenol F, bisphenol E, bisphenol B, bisphenol Z, bisphenol AF, bisphenol AP, tetrabromobisphenol A and bisphenol P – in solid foodstuffs, and particularly in ready-made meals.

Extraction was carried out by ultrasound-assisted extraction after sample disruption with sand. A selective solid-phase extraction procedure was then applied to reduce potential matrix interferences. Derivatization of bisphenols with pyridine-3-sulfonyl chloride increased their ionization efficiency by electrospray ionization. Validation of the proposed method was performed in terms of selectivity, matrix effects, linearity, precision, measurement uncertainty, trueness and limits of detection. Satisfactory repeatability and intermediate precision were obtained; the related relative standard deviations were ≤7.8% and ≤10%, respectively. The relative expanded uncertainty ($k = 2$) was below 17% for all bisphenol analogs and the trueness of the method was demonstrated by spike recovery experiments. Low limits of detection, in the range from 0.025 μg kg^{-1} to 0.140 μg kg^{-1}, were obtained for all compounds. To demonstrate the applicability of the proposed method, it was eventually applied to several ready-made meals purchased from different supermarkets in Belgium.

1. Introduction

Modern lifestyle has significantly changed eating habits worldwide, leading to an increase in demand of ready-made meals over the last few years [1]. Many consumers work longer hours and spend less time planning and preparing meals, so they seek convenient alternatives that allow them to maximize their leisure time [2]. Ready-made meals can be defined as pre-prepared, chilled or frozen meals that require no extra ingredients and need only minimal preparation before consumption. Most of times, they are packaged in disposable plastic trays and only require heating (usually in their own packaging) before being served. Due to their growing consumption, this kind of convenience food is a major dietary component for many people in today's society, thus potentially representing an important route of human exposure to several food contaminants [3].

Among them, bisphenol A (BPA), a chemical used in the manufacture of polycarbonate plastics and epoxy resins, is attracting growing attention due to its extensive use in a large variety of applications including food and liquid containers, kitchenware, inner linings of metal cans and bottle tops, surface coatings, toys, medical devices, dental fillings and cash register receipts, among others [4]. In the European Union (EU), BPA is permitted as a monomer in food contact materials under Commission Regulation (EU) No 10/2011 [5], relating to plastic materials and articles intended to come into contact with foodstuffs, with a specific migration limit

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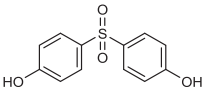
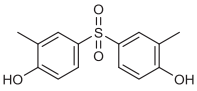
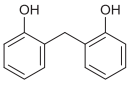
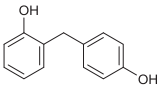
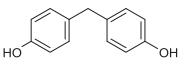
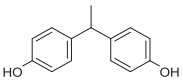
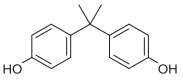
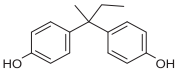
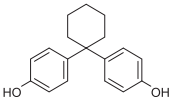
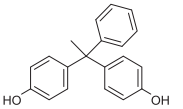
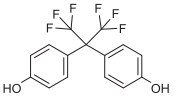
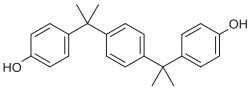
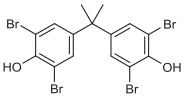
of 0.6 mg kg^{-1}. However, the use of BPA in infant feeding bottles has been recently banned [6]. These restrictions have forced the plastic industry to look for alternative chemicals to replace BPA, most of them belonging to the same chemical group of *p,p′*-bisphenols (Table 1). Among these structural analogs, bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB) and bisphenol AF (BPAF) are apparently the major BPA replacements [7,8].

The determination of these emerging contaminants in foodstuffs requires the development and validation of appropriate and robust analytical methods. To date, nevertheless, few methods have been developed for the analysis of BPA analogs in food samples in general, and in ready-made meals, in particular. The higher degree of industrial processing/manipulation that these ready-to-eat meals normally undergo (storage, cooking, packaging, chilling, etc.) may considerably increase the risk for contamination with bisphenols. From the analytical point of view, this kind of samples represents a challenging problem, not only because of the enormous variety of different ready-made meals available on the market, but also because of the high complexity of these composite dishes. An additional difficulty might be observed when mixing a contaminated ingredient with several less/non-contaminated components of the whole meal. Therefore, low limits of detection (LODs) are necessary to overcome this dilution effect.

Solid–liquid extraction (SLE) is the most common technique for the extraction of BPA from solid foodstuffs [4], and it has also been applied for the extraction of some BPA analogs [8,9]. The combination of SLE with a preconcentration step by dispersive liquid–liquid microextraction has been reported for the extraction of BPA and BPB from canned vegetables, fruits and seafood [10,11]. Viñas et al. [12] developed a method based on SLE followed by

Table 1
Physicochemical properties and structures of the studied bisphenols.

Compound	Acronym	CAS number	Monoisotopic mass (*u*)	Log K_{ow}	pK_a	Structure
Bisphenol S	BPS	80-09-1	250.03	2.32	7.42–8.03	
4,4′-Sulfonylbis(2-methylphenol)	DMBPS	16346-97-7	278.06	3.35	7.79–8.40	
2,2′-Bisphenol F	2,2′-BPF	2467-02-9	200.08	3.46	8.26–11.90	
2,4′-Bisphenol F	2,4′-BPF	2467-03-0	200.08	3.46	9.80–10.44	
4,4′-Bisphenol F	4,4′-BPF	620-92-8	200.08	3.46	9.84–10.45	
Bisphenol E	BPE	2081-08-5	214.10	3.74	9.81–10.42	
Bisphenol A	BPA	80-05-7	228.12	4.04	9.78–10.39	
Bisphenol B	BPB	77-40-7	242.13	4.49	9.77–10.38	
Bisphenol Z	BPZ	843-55-0	268.15	4.91	9.76–10.37	
Bisphenol AP	BPAP	1571-75-1	290.13	5.18	9.66–10.27	
Bisphenol AF	BPAF	1478-61-1	336.06	4.77	9.13–9.74	
Bisphenol P	BPP	2167-51-3	346.19	6.72	9.78–10.38	
Tetrabromobisphenol A	TBBPA	79-94-7	539.76	7.12	6.57–7.18	

in situ acetylation/solid-phase microextraction for the determination of BPA and BPS in canned vegetables. Other extraction techniques such as ultrasound-assisted extraction (USAE) [13] and supramolecular solvent extraction [14] have been recently reported for the extraction of some bisphenols from canned foods.

Regarding the detection technique, most of methods are based on gas chromatography coupled to mass spectrometry (GC–MS) after a derivatization step with acetic anhydride [9–12] or bis(trimethylsilyl)trifluoroacetamide [12], although some authors have also used liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) [8] or fluorescence detection [14].

The aim of the current work was to develop and validate a robust method based on stable-isotope dilution (SID) liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the determination of BPA and its main analogs – BPS, DMBPS, BPF, BPE, BPB, BPZ, BPAF, BPAP, TBBPA and BPP (Table 1) – in solid foodstuffs in general, and particularly in ready-made meals. Extraction of bisphenols was carried out by USAE after an initial sample disruption step with sand. Due to the complexity of the analyzed samples, solid-phase extraction (SPE) was applied to reduce potential matrix interferences. Derivatization of bisphenols with pyridine-3-sulfonyl (PS) chloride increased their ionization efficiency by electrospray ionization (ESI), thus improving the LODs. Validation of the proposed method was performed in terms of selectivity, linearity, precision, measurement uncertainty, trueness, LODs and matrix effects. Several ready-made meals purchased from different supermarkets in Belgium were finally analyzed in order to demonstrate the applicability of the method.

2. Materials and methods

2.1. Standards, reagents and materials

Bisphenol A (≥99%), bisphenol AF (97%), bisphenol AP (99%), 2,2′-bisphenol F (>98%), 4,4′-bisphenol F (98%), bisphenol P (99%), bisphenol S (98%), bisphenol Z (98%), 4,4′-sulfonylbis(2-methylphenol) (97%), tetrabromobisphenol A (≥97%) and pyridine-3-sulfonyl chloride (95%) were purchased from Sigma–Aldrich (Diegem, Belgium). Bisphenol B (>98%), bisphenol E (>98%) and 2,4′-bisphenol F (>98%) were obtained from TCI (Zwijndrecht, Belgium). Bisphenol A-$^{13}C_{12}$ (99.2% ^{13}C, 98% chemical purity), 4,4′-bisphenol F-D_{10} (96.8% D, 98% chemical purity) and bisphenol S-$^{13}C_{12}$ (99.6% ^{13}C, 97% chemical purity) were purchased from Toronto Research Chemicals (North York, Canada). Bisphenol AF-3,3′,5,5′-D_4 (99.4% D, 99% chemical purity) was obtained from C/D/N isotopes (Pointe-Claire, Canada) and $^{13}C_{12}$-tetrabromobisphenol A (99% ^{13}C, 50 mg L^{-1} in methanol) was from Cambridge Isotope Laboratories (Andover, MA, USA). Chemical structures, octanol-water partition coefficients (log K_{ow}) and pK_a values of the analyzed compounds are shown in Table 1. ChemAxon's Calculator Plugins were used for structure property prediction and calculation [15].

Individual stock solutions (ca. 1000 mg L^{-1}) were prepared in methanol by accurately weighing amounts between 20 and 30 mg of each analyte on an analytical balance ME235S from Sartorius (Goettingen, Germany). A mixture of them and the subsequent working standard solutions were made by appropriate dilution in methanol and then stored in amber glass vials at −20 °C.

All organic solvents (acetonitrile, ethyl acetate, *n*-hexane and methanol) were HPLC or LC–MS grade and all other chemicals were of analytical reagent grade. Ultrapure water was produced using a Milli-Q Gradient water purification system from Merck Millipore (Bedford, MA, USA). Acetic acid (100%), formic acid (98–100%), hydrochloric acid (37%), ammonium hydroxide (28–30%), sodium hydroxide, anhydrous sodium sulfate (0.63–2.0 mm) and anhydrous sodium carbonate were purchased from Merck (Darmstadt, Germany). Sand (50–70 mesh), Florisil (60–100 mesh), neutral alumina Supelclean Alumina-N (60–325 mesh), ethylenediamine-N-propyl bonded silica gel (primary secondary amine) Supelclean PSA (PSA, 50 μm) and octadecyl-bonded silica gel Discovery DSC-18 (C18, 50 μm) were acquired from Sigma–Aldrich. Normal-phase sorbents were activated at 130 °C for 12 h. After activation, they were allowed to cool down in a desiccator before being used. C18 and PSA sorbents were used as received.

SPE cartridges Supelclean PSA (500 mg, 6 mL) were purchased from Sigma–Aldrich and Strata-X (200 mg, 6 mL) were obtained from Phenomenex (Utrecht, Netherlands). Regenerated cellulose membrane syringe filters (13 mm, 0.2 μm) were purchased from Grace (Lokeren, Belgium).

2.2. Samples

All food samples were purchased between February and December 2014 from supermarkets located in Belgium and belonging to three major European supermarket chains. The ready-made meals investigated were private labels of the supermarkets, chilled or sterilized (long shelf life at room temperature). The meals comprised a main dish designed to replace the main course of a homemade meal, typically a meat or fish portion, a starchy component (rice/potatoes/pasta), a vegetable portion and/or a sauce. Frozen pizza and canned ravioli were also included in this study as they are among the most consumed ready-made meals worldwide. Most of the ready-made meals were heated in a microwave oven in their original plastic trays, as specified on the label by the manufacturers including time (3–6 min) and power (650–850 W) settings. Pizza was baked in an oven at 180 °C for 10 min and ravioli were removed from the can and then microwave heated in a glass dish. For method validation, a blank composite meal was prepared in an experimental kitchen avoiding any plasticware during the cooking process. In order to obtain a sample representative of a typical whole meal, its composition was selected following recommended dietary reference intakes for macronutrients [16]. The composite sample consisted of chicken breast (65 g, pan grilled), short-grain white rice (356 g, boiled), green apples (200 g, with peel) and extra virgin olive oil (30 g).

After reheating/cooking, food samples were homogenized using a stainless steel blender and then stored in glass bottles at −20 °C until processing. For the preparation of spiked samples, a mixed standard solution of bisphenols in methanol was added to an accurately weighed amount of homogenized sample and allowed to stand at room temperature until complete evaporation of the solvent (2–3 h) before extraction.

2.3. Ultrasound-assisted extraction

Food samples (ca. 2 g) were accurately weighed in an aluminum dish and spiked with 2.5 ng of isotope-labeled standards in methanol (100 μL, 25 μg L^{-1}). After solvent evaporation, samples were disrupted with 1 g of sand and 1 g of anhydrous sodium sulfate in a porcelain mortar with a pestle, until a homogenous mixture was obtained. The homogenate was transferred to a 50 mL polypropylene centrifuge tube and 6 mL acetonitrile/methanol (80:20, v/v) were added. After vortex shaking for 10 s, the tube was immersed in an ultrasonic water bath Branson 2510 from Emerson (Dietzenbach, Germany). Extractions were performed at 40 kHz of ultrasound frequency at 30 ± 3 °C for 20 min. The resulting slurry was centrifuged at 3000 RCF for 5 min at 10 °C (Eppendorf 5810R, Hamburg, Germany) and the supernatant was collected; the extraction procedure was repeated once more and both supernatants were combined in a 16 mL glass test tube. The extract was evaporated to near dryness at 35 °C under a nitrogen flow in a sample concentrator Techne FSC400D (Bibby Scientific, Roissy, France), then reconstituted in 6 mL of ethyl acetate/*n*-hexane (50:50, v/v)

and ca. 100 mg of anhydrous sodium sulfate were added for further drying.

2.4. SPE clean-up and derivatization

Sample clean-up was carried out by SPE using Supelclean PSA cartridges (500 mg, 6 mL). The optimized protocol involved conditioning the cartridges with 6 mL of methanol followed by 6 mL of methanol/ethyl acetate (50:50, v/v) and 6 mL of ethyl acetate. The extract (6 mL) was loaded, rinsed with 6 mL of ethyl acetate and eluted with 10 mL of a mixture of methanol/ethyl acetate/acetic acid (20:78.5:1.5, v/v/v).

The bisphenols were then derivatized with PS chloride following the conditions recently reported by Regueiro et al. [17]. Briefly, the SPE eluate was evaporated to dryness at 35 °C under a nitrogen steam, reconstituted in 200 μL of sodium carbonate buffer (50 mmol L^{-1}, pH 9.8) and 200 μL of derivatization solution (4 mg mL^{-1} of PS chloride in acetonitrile) were added. After vortex shaking for 10 s, the reaction mixture was placed in a dry block heater Techne DB100/2 (Bibby Scientific) at 70 °C for 15 min. Reaction was stopped by cooling down on ice and 100 μL of formic acid solution 1 mol L^{-1} were added. The extract was passed through 0.20 μm regenerated cellulose syringe filter and stored in amber glass vials at −20 °C until analysis.

2.5. LC–MS/MS analysis

Sample analyses were performed using an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) consisting of a binary pump, a vacuum degasser, an autosampler, and a column oven, coupled to a triple quadrupole mass spectrometer Waters Micromass Quattro Ultima PT (Waters, Milford, MA, USA) equipped with an ESI source.

Chromatographic separation was carried out on a pentafluorophenylpropyl Ascentis Express F5 column (100 mm × 2.1 mm, 2.7 μm) from Sigma–Aldrich, equipped with a F5 guard column (5 mm × 2.1 mm, 2.7 μm) and maintained at 25 °C. Mobile phases A and B were water/formic acid (99.8:0.2, v/v) and acetonitrile/water/formic acid (97.8:2:0.2, v/v/v), respectively. The following linear gradient was used: 0 min, 45% B; 0.5 min, 45% B; 9.5 min, 75% B; 10.5 min, 98% B; 12.0 min, 98% B; 12.5 min, 45% B and 18 min, 45% B. The flow rate was set to 240 μL min^{-1}, and the injection volume was 10 μL. To prevent salts from entering the ion source, the LC eluate was diverted to waste during the first 4.5 min of the chromatographic run. During the method development, an XBridge C18 column (50 mm × 2.1 mm, 2.5 μm) from Waters and an Ascentis Express C18 column (100 mm × 2.1 mm, 2.7 μm) from Sigma–Aldrich were also employed.

The mass spectrometer was operated in the positive ESI mode under the following specific conditions: capillary voltage 3.60 kV, cone voltage 70 V, desolvation temperature 350 °C, source temperature 130 °C, cone gas flow 80 L h^{-1} and desolvation gas flow 750 L h^{-1}. Nitrogen (boil-off) was employed as nebulizer, desolvation and cone gas. The RF lens voltages 1 and 2 were set at 10 and 0.4 V, respectively. The multiplier voltage was 650 V and the ion energies 1 and 2 were both 0.5 V. The entrance and exit voltages were −2 and 1 V, respectively. Analyte detection was performed in multiple reaction monitoring (MRM) mode using Argon as collision gas at a pressure of 4.5×10^{-3} mbar. Instrument control and data acquisition were performed with MassLynx v4.0 software from Waters.

2.6. Statistical analysis

Statistical calculations were made using the software package Statgraphics Centurion XV (Statpoint Technologies, Herndon, VA, USA). Unless otherwise specified, data are presented as the mean ± standard deviation (SD) and a 0.05 significance level was used.

3. Results and discussion

3.1. Preliminary experiments

In order to achieve LODs low enough to overcome the dilution effect that can be observed when mixing contaminated ingredients with less/no contaminated ones such as expected for composite meals, highly sensitive methodologies are required. Over the last years, LC–ESI-MS/MS has become one of the most valuable analytical techniques in food analysis due to its sensitivity and selectivity for a wide array of compounds. However, most of the studied bisphenols show acid-base properties (indicated by high pK_a values; Table 1) that limit their ionization in ESI mode under acidic to neutral conditions.

Some authors proposed the use of ammonia (0.05–0.1%, v/v) as a mobile phase additive under reversed-phase conditions to improve the ionization efficiency of BPA in negative ESI mode [18,19]. However, in the current study the addition of 0.1% ammonia (pH 10) to mobile phases consisting of methanol/water did not improve signals significantly if compared to the signals obtained in the absence of ammonia under elsewise identical conditions (data not shown). In addition, under these basic conditions, the most acidic compound BPS was completely unretained by the Xbridge C18 column (50 mm × 2.1 mm, 2.5 μm), eluting at the column void volume even at very low organic mobile phase percentages (2% methanol). Recently, several studies have also reported a negative effect of ammonia on the ionization of BPF, BPAF and TBBPA [19,20].

Chemical derivatization using PS chloride has been very recently applied for the determination of BPA in different kind of biological matrices showing successful results with regard to both sensitivity and specificity [21–23]. This derivatization reagent presents a major advantage as compared to the widely used dansyl chloride, since MS/MS transitions involve analyte-specific product ions rather than reagent-specific product ions [17,24]. In this way, interferences arising from matrix components, which are of special concern when analyzing complex samples, are reduced.

MRM conditions were optimized by post-column infusion of the resulting derivatized standard solutions. Although in low-resolution MS, the obtained product ions were coincident with those analyte-specific product ions reported in a previous work in a hybrid ion trap-Orbitrap mass spectrometer [17]. Two MS/MS ion transitions were monitored for each compound; the most intense transition was used for quantification, while the other one was employed for identification (Table 2). Confirmation was accomplished by comparing the quantifier-to-qualifier transition ratios in samples to those of the calibration standards within the maximum permitted tolerances in accordance with Commission Decision 2002/657/EC [25], which was used as a guide.

Unlike BPA, BPF is normally used as a technical mixture of three isomers, 2,2′-, 2,4′- and 4,4′-dihydroxydiphenylmethane, in the approximate ratios of 15, 50 and 35%, respectively [9]. Kitamura et al. [26] reported that the presence of at least one 4-hydroxyl group is essential for hormone-like, estrogenic and antiandrogenic activities of bisphenol analogs. Therefore, different toxicities are expected for these BPF isomers, which highlights the importance of a separate quantification of these analogs.

To the best of our knowledge, all available information on BPF toxicity refers to 4,4′-BPF, which is used as a model compound due to its structural similarity with BPA [26–29]. The MS/MS fragmentation of BPF isomers produced spectra with identical fragmentation patterns, presenting only slight differences in

Table 2
Specific MRM conditions for determination of bisphenols after derivatization with PS chloride.

Compound	t_R (min)	Parent ion	Cone (V)	MRM1 (*m/z*)	CE1 (eV)	MRM2 (*m/z*)	CE2 (eV)	T1/T2 ± tol.[b]
BPS-diPS	5.43	$[M+H]^+$	60	532.9 > 327.1	23	532.9 > 391.1	23	1.7 ± 0.3
BPS-$^{13}C_{12}$-diPS[a]	5.43	$[M+H]^+$	60	544.9 > 339.1	23	544.9 > 403.1	23	1.7 ± 0.3
2,2′-BPF-diPS	5.55	$[M+H]^+$	70	483.2 > 199.0	25	483.2 > 277.2	25	1.5 ± 0.3
2,4′-BPF-diPS	6.21	$[M+H]^+$	70	483.2 > 199.0	25	483.2 > 277.2	25	1.9 ± 0.4
4,4′-BPF-D_{10}-diPS[a]	6.51	$[M+H]^+$	70	493.2 > 209.0	25	493.2 > 287.2	25	1.1 ± 0.2
4,4′-BPF-diPS	6.55	$[M+H]^+$	70	483.2 > 199.0	25	483.2 > 277.2	25	1.1 ± 0.2
DMBPS-diPS	6.74	$[M+H]^+$	60	561.3 > 355.1	23	561.3 > 419.1	23	1.6 ± 0.3
BPE-diPS	7.06	$[M+H]^+$	70	497.3 > 340.2	28	497.3 > 276.1	35	1.8 ± 0.4
BPA-diPS	7.62	$[M+H]^+$	70	511.3 > 354.2	28	511.3 > 290.1	35	2.2 ± 0.6
BPA-$^{13}C_{12}$-diPS[a]	7.62	$[M+H]^+$	70	523.2 > 366.2	28	523.2 > 302.1	35	2.2 ± 0.6
BPB-diPS	8.19	$[M+H]^+$	70	525.3 > 354.2	28	525.3 > 290.1	28	1.8 ± 0.4
BPZ-diPS	8.65	$[M+H]^+$	70	551.3 > 267.2	30	551.3 > 248.0	32	4.4 ± 1.1
BPAP-diPS	8.86	$[M+H]^+$	70	573.3 > 416.2	30	573.3 > 196.0	32	3.3 ± 0.8
BPAF-diPS	9.36	$[M+H]^+$	70	619.1 > 344.1	35	619.1 > 408.1	32	1.5 ± 0.3
BPAF-D_4-diPS[a]	9.36	$[M+H]^+$	70	623.1 > 348.1	35	623.1 > 412.1	32	1.5 ± 0.3
TBBPA-diPS	9.41	$[M+H]^+$	70	826.7 > 605.6	45	826.7 > 620.6	32	1.2 ± 0.2
TBBPA-$^{13}C_{12}$-diPS[a]	9.41	$[M+H]^+$	70	838.7 > 617.6	45	838.7 > 632.6	32	1.2 ± 0.2
BPP-diPS	10.31	$[M+H]^+$	70	629.4 > 276.1	28	629.4 > 134.0	35	2.0 ± 0.4

MRM1: quantifier transition; MRM2: qualifier transition; CE: collision energy.
[a] Isotope-labeled standard.
[b] Quantifier-to-qualifier transition ratios and tolerances for positive identification.

abundance of diagnostic ions; therefore, the chromatographic separation of the isomers became necessary in order to avoid biased estimations of the contents of 4,4′-BPF in food samples. A core-shell particles column Ascentis Express C18 (100 mm × 2.1 mm, 2.7 μm) was initially tested under different binary gradients with water/methanol or water/acetonitrile containing formic acid (0.1–0.3%, v/v) as a modifier. However, none of these conditions allowed achieving a good separation between the PS-derivatives of 2,4′-BPF and 4,4′-BPF, which showed a resolution value (Rs) below 1 (Fig. 1a). A similar approach was then followed using a pentafluorophenylpropyl (PFPP) column Ascentis Express F5 (100 mm × 2.1 mm, 2.7 μm). The PFPP stationary phase presents unique selectivity due to the presence of strongly electronegative fluorine atoms on the phenyl ring. The resulting electron-deficient π system is able to interact in a stronger manner with electron-rich π systems like those in phenols. Interactions through other mechanisms such as dipole–dipole and hydrogen bonding are also possible [30]. The best separation was obtained with water and acetonitrile as mobile phases, both containing 0.2% formic acid (v/v). By applying this column under the optimized chromatographic conditions detailed in Section 2, baseline separation (Rs ≥ 1.5) was obtained for all three BPF isomers (Fig. 1b) without hampering chromatography of all other studied bisphenols (Fig. 2).

3.2. Extraction

One of the major difficulties when analyzing organic compounds in food samples is the co-extraction of matrix components. The presence of matrix interferents, such as lipids and proteins, in sample extracts can negatively affect both the chromatographic

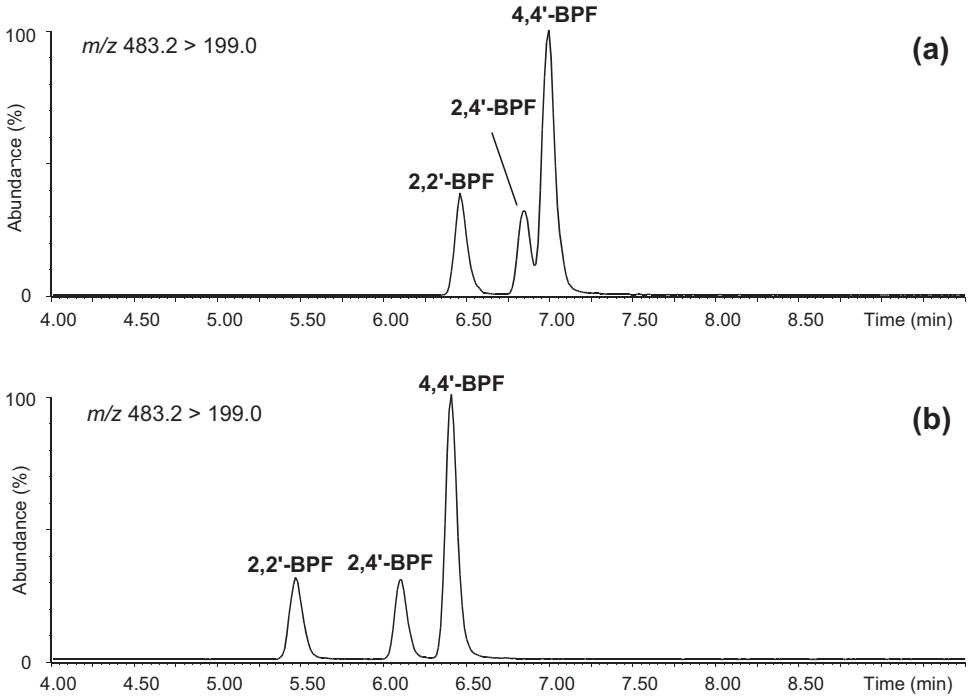


Fig. 1. Chromatographic separation of a standard mixture of BPF isomers (2,2′-BPF, 10 μg L^{-1}; 2,4′-BPF, 10 μg L^{-1}; 4,4′-BPF, 50 μg L^{-1}) using C18 (a) and PFPP (b) stationary phases.

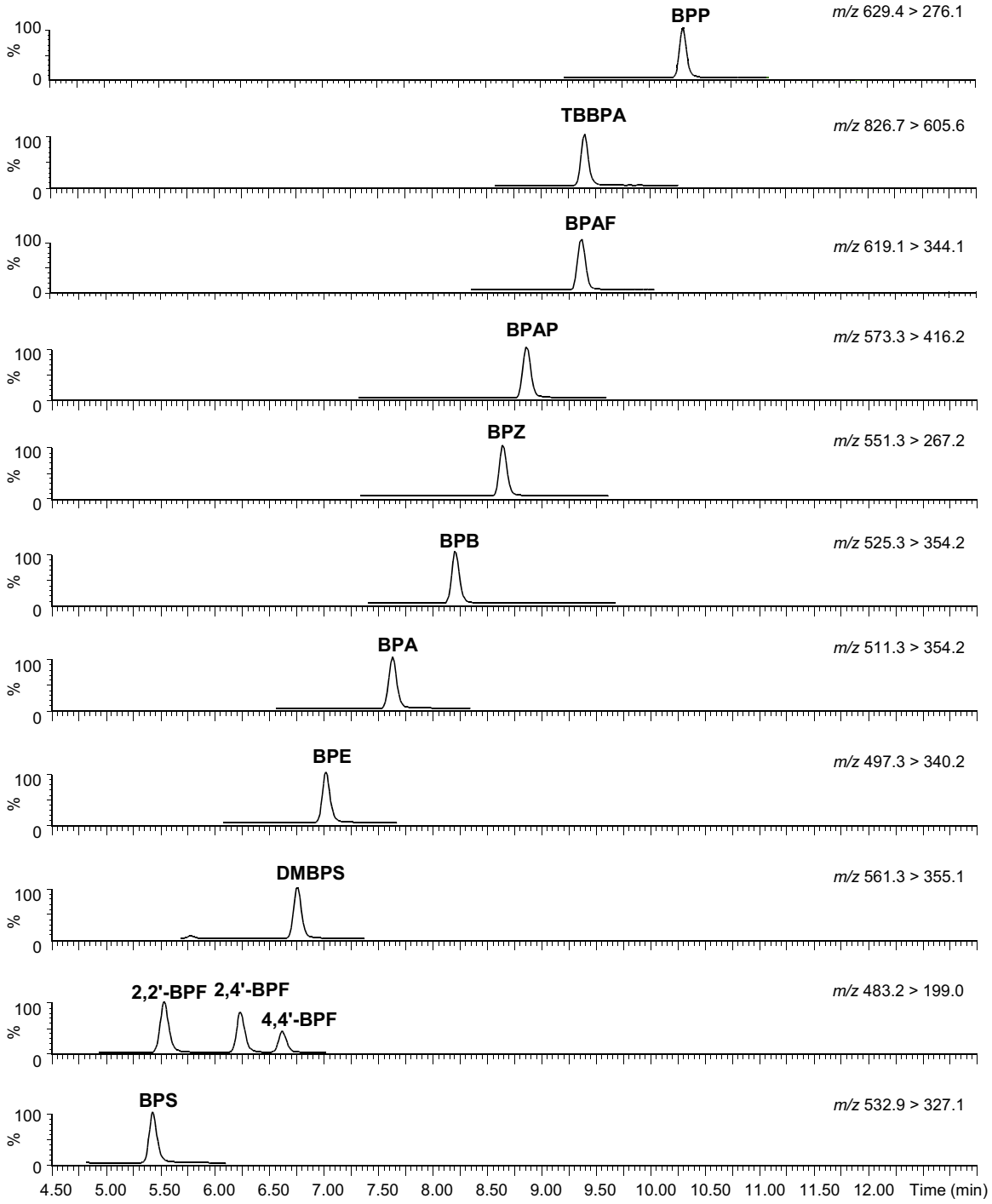


Fig. 2. MRM chromatograms obtained for a standard mixture of bisphenols (10 μg L^{-1}).

separation and the detection of the analytes. Therefore, a key issue was to minimize the lipid and protein content of the extracts without reducing the efficiency of the extraction process.

The fine dispersion of the sample prior to USAE can improve the interactions between sample and extraction solvent, thus increasing the efficiency and reproducibility of the process. Sample dispersion with suitable sorbents has also been applied in other extraction techniques, such as pressurized liquid extraction (PLE), to produce selective extractions of different contaminants in complex samples [31,32].

Therefore, the effect of several reversed- and normal-phase sorbents (C18, PSA, neutral alumina, Florisil and sand) on the extraction of analytes was investigated using both acetonitrile and acetonitrile/methanol (80:20, v/v) as extraction solvents (Fig. 3). These experiments were performed with portions of a homogenized ready-made meal sample (5.6% fat, 8.1% protein, 9.1% carbohydrates and 1.4% fiber; values taken from the nutrient declaration on the label) spiked with all compounds to a level of 5.0 μg kg^{-1}. As can be seen, the best results were obtained when the matrix dispersion was carried out with sand, showing recoveries between 79% and 101% for all the studied bisphenols. Slightly higher responses were observed when acetonitrile/methanol was used. Due to their phenolic nature, bisphenols are able to readily establish hydrogen bonds with active sites in the surface of sand.

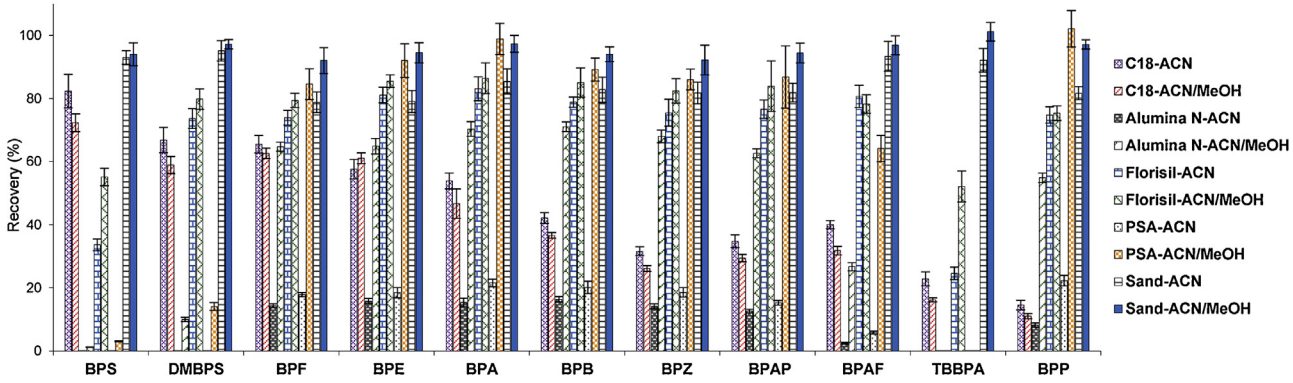


Fig. 3. Effect of different combinations of dispersing materials and solvents on the recovery of bisphenols.

The presence of a polar protic solvent, such as methanol, usually disrupts these bonds, thus increasing extraction efficiency. For the reversed-phase sorbent C18, recoveries clearly decreased with increasing lipophilicity of the target compounds, even using 100% acetonitrile recoveries ranged from around 75% for the most polar compound BPS ($\log K_{ow} = 2.32$) to below 20% for the highly lipophilic BPP ($\log K_{ow} = 6.72$). For alumina and PSA, extraction efficiencies were in all cases below 20% when acetonitrile was used. Although for most of the bisphenols recoveries increased significantly with acetonitrile/methanol, BPS and TBBPA could not be recovered most likely due to their stronger interactions with these sorbents. The combination of Florisil with acetonitrile/methanol provided satisfactory results for most of the bisphenols, but not for BPS and TBBPA with recoveries still below 55%.

Although sand cannot be strictly considered as a dispersing agent since it lacks absorptive/adsorptive properties to yield a real dispersion of the analytes, it acts as an abrasive solid support material. Thus, sand produces shearing forces during the blending process that induce the disruption of the sample architecture, enabling consequently a more efficient extraction [33]. For instance, some authors have successfully applied sand as a disruption agent in the extraction of phenolic compounds and sulfonamides from vegetables [33,34] and biological samples [35], respectively. In addition, the use of sand significantly lowers the cost of the analytical procedure as compared to other more expensive dispersing agents.

To further evaluate the effect of sample disruption on the extraction efficiency, two different ready-made meal samples, S1 (9.7% fat, 6.5% protein, 8.5% carbohydrates and 1.5% fiber) and S2 (1.4% fat, 9.5% protein, 9.7% carbohydrates and 1.2% fiber), spiked to a level of 5.0 μg kg^{-1} were ultrasound extracted using acetonitrile/methanol (80:20, v/v) with and without the previous sample disruption step with sand (Fig. 4). For the less fatty sample S2, no significant differences were observed for any of the bisphenols. However, recoveries for the most lipophilic bisphenols (BPZ, BPAP, TBBPA and BPP) were statistically significantly lower ($p < 0.05$) in sample S1 when the extraction was carried out without sample disruption. These results seem to indicate that the sand sample disruption allows improving the efficiency of USAE of lipophilic bisphenols from fatty samples. Very recently, Yang et al. [13] developed a method for the extraction of BPA, BPS, BPF, BPB, BPAF, TBBPA and tetrachlorobisphenol A from canned foods by USAE with acetonitrile followed by delipidation by liquid–liquid extraction (LLE) with *n*-hexane. Following this procedure, the authors reported recoveries significantly lower in canned fish, especially for TBBPA (57 ± 14% to 67 ± 8%), likely due to the less efficient extraction procedure and the losses during the LLE step.

It is worthy to remark that the extraction method proposed in the present work is compatible with fresh samples, which constitutes a substantial saving in total analysis time as compared to other methods based on the use of freeze-dried samples [8].

3.3. Clean-up conditions

Due to the complexity of the ready-made meal samples, a clean-up step by SPE was needed. Two different SPE sorbents were tested, namely ethylenediamine-N-propyl phase Supelclean PSA and the polymeric reversed-phase Strata-X. These experiments were performed with portions of the ready-made meal sample S1 (the most complex one) spiked with all compounds to a level of 5.0 μg kg^{-1}. After extraction following the previously discussed conditions, the extracts were concentrated to dryness under a gentle steam of nitrogen and then reconstituted in a suitable solvent prior to the SPE. For the polymeric phase, the residue was

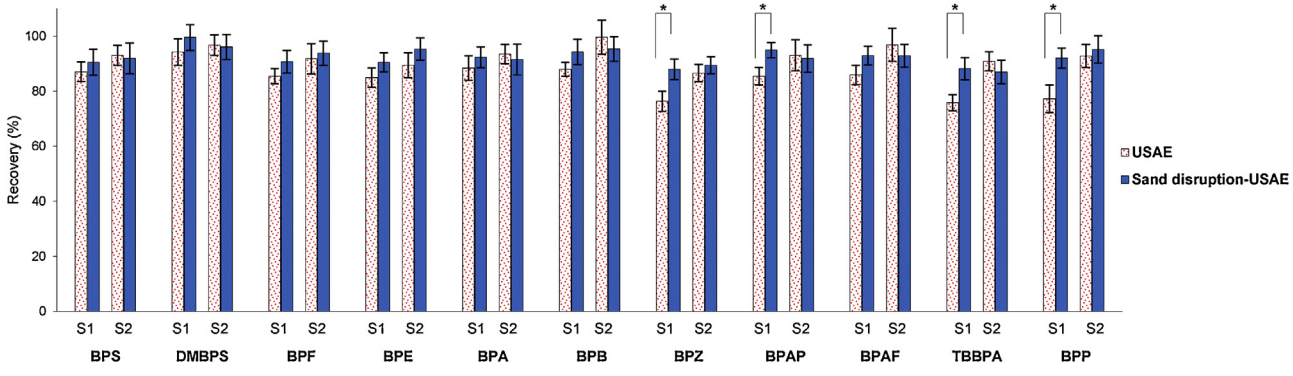


Fig. 4. Comparison of the extraction efficiency of USAE and USAE preceded by sand sample disruption for two ready-made meals (S1, 9.7% fat and S2, 1.4% fat) spiked at 5.0 μg kg^{-1}. * Statistically significant ($p < 0.05$).

dissolved in 10 mL of 1% formic acid in water (v/v), which produced a very turbid dispersion likely due to the high lipid content. The dispersion completely clogged the SPE cartridge during the loading step, making this approach unsuitable for the clean-up of this kind of samples. The PSA sorbent is normally recommended for the clean-up of complex samples, especially due to its ability to retain fatty acids and other matrix interferences, such as organic acids, and some polar pigments and sugars [36,37]. For this phase, the extract residue was reconstituted in ethyl acetate/*n*-hexane (50:50, v/v) and any trace of water was removed by addition of anhydrous sodium sulfate. Under these conditions, PSA should retain bisphenols mainly through dipole–dipole interactions and hydrogen bonding with the hydroxyl moieties. After loading the sample extract, the cartridge was rinsed with 6 mL of ethyl acetate, without losing any of the bisphenols. Elution of bisphenols from the SPE cartridge was studied with different solvents with the goal of obtaining a high level of selectivity paired with good recoveries. Elution of bisphenols was assessed with each 10 mL of the following eluents: methanol, methanol/acetic acid (98.5:1.5, v/v), ethyl acetate/acetic acid (98.5:1.5, v/v) and methanol/ethyl acetate/acetic acid (20:78.5:1.5, v/v/v).

As shown in Fig. 5, methanol provided recoveries above 91% for all bisphenols except BPS and TBBPA, which were completely retained on the PSA sorbent. This result, along with those observed during the extraction optimization, confirmed that these two compounds are more strongly retained on PSA than the rest of the studied bisphenols. When elution was carried out with methanol containing 1.5% acetic acid (v/v), quantitative recoveries were obtained for all the analytes, indicating that the presence of this acid is able to disrupt the interactions of BPS and TBBPA with the ethylenediamine-N-propyl phase. This behavior suggests that for BPS and TBBPA, the most acidic bisphenols, retention on the PSA sorbent is not only based on hydrogen bonding and dipole–dipole interactions, but also on ionic interactions. Although in non-aqueous media PSA normally acts as a normal-phase sorbent, it can also behave as a weak anion exchanger (WAX), able to retain analytes holding a net negative charge. Thus, it was hypothesized that under the studied conditions BPS and TBBPA are at least partially ionized. The addition of acetic acid neutralizes them, allowing them to elute from the cartridge.

When acetic acid was added to ethyl acetate instead of methanol, recoveries dropped to below 3% for all studied bisphenols, which indicates the need of a protic solvent to disrupt the hydrogen bonds between the analytes and the sorbent moieties.

The mix of methanol/ethyl acetate/acetic acid (20:78.5:1.5, v/v/v) provided also quantitative recoveries for all studied bisphenols, but the resulting extracts were apparently much cleaner compared to methanol/acetic acid (98.5:1.5, v/v), rendering clearer, yellowish extracts. Thus, the decrease in the percentage of methanol seems to reduce the co-elution of polar matrix interferences from the PSA sorbent. However, percentages of methanol below 20% resulted in incomplete elution of some bisphenols (data not shown), so this elution mixture was finally selected for the SPE procedure.

3.4. Method performance

Validation of the proposed method was performed based on the recommendations of the Eurachem guide on analytical method validation [38] and Commission Decision 2002/657/EC establishing criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories [25]. Due to the ubiquitous presence of BPA, it was not possible to obtain procedural blanks completely free of this compound, so the background level was calculated for every batch of samples and then deducted from the BPA concentration in the analyzed samples. For spiking experiments, a blank in-house prepared composite meal sample (see Section 2.2 for details) was used.

3.4.1. Linearity

The linearity of the method was tested using standard solutions at eight concentration levels evenly distributed over the range of 0.4–160 $\mu g\,L^{-1}$ (Table 3). Each concentration level was analyzed at least in triplicate. Calibration curves were constructed using the ratios of the peak area of the compounds to the peak area of the isotope-labeled internal standards. Determination coefficients (R^2) greater than 0.998 were obtained for all compounds using weighted ($1/x^2$) linear calibration curves. The lack-of-fit (LOF) test was applied to statistically decide whether the selected linear model was adequate to describe the experimental data. The test compares the variability of the proposed model residuals to the variability between observations at replicate values of the independent variable. Results of the LOF test for the calibration range considered, at a confidence level of 95% are also shown in Table 3. Since *p*-values were greater than 0.05 for all compounds, the linear regression models appear to adequately fit the data.

3.4.2. Selectivity

The selectivity of the method was assessed via the analysis of procedural blank samples, blank ready-made meal samples and different ready-made meal samples spiked at 1.0 $\mu g\,kg^{-1}$. MRM chromatograms obtained for quantifier and qualifier MS/MS transitions were checked for co-eluting interferents at the retention

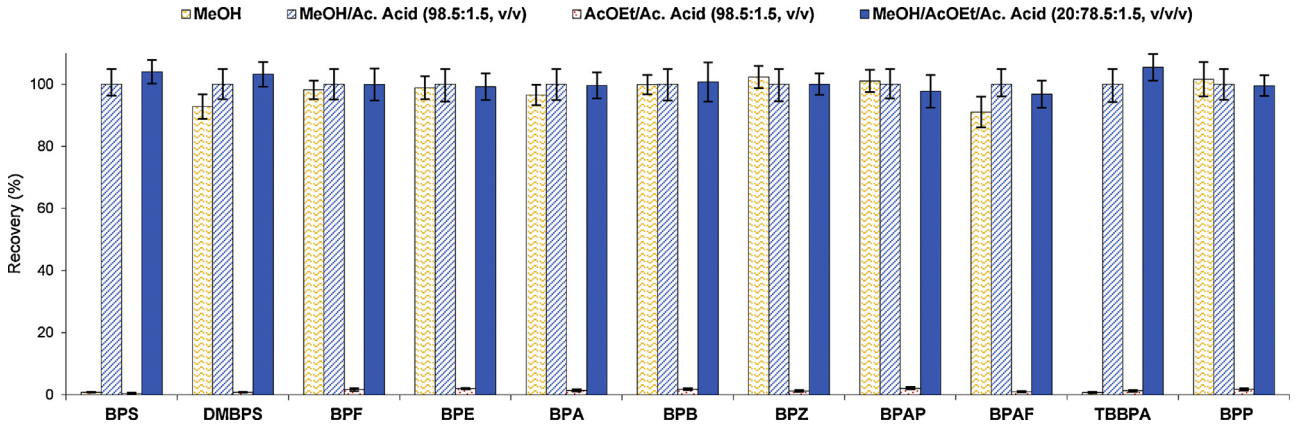


Fig. 5. Effect of different elution solvents on the recovery of bisphenols during SPE with PSA.

Table 3
Linearity evaluation of the proposed method.

Compound	Calibration range ($\mu g\,L^{-1}$)	Intercept	Slope	R^2	LOF (p-value)
BPS	0.461–142.035	−0.0025	1.0299	0.9996	0.7056
DMBPS	0.472–145.355	0.0028	0.1880	0.9990	0.1799
2,2′-BPF	0.446–137.362	−0.0167	1.6512	0.9986	0.1548
2,4′-BPF	0.472–145.211	−0.0062	1.1323	0.9985	0.2662
4,4′-BPF	0.465–143.165	−0.0016	1.1012	0.9985	0.9961
BPE	0.463–142.673	−0.0017	0.6606	0.9998	0.7903
BPA	0.520–160.047	0.0162	0.7409	0.9999	0.8637
BPB	0.475–146.301	−0.0010	0.8128	0.9986	0.062
BPAP	0.496–152.872	0.0094	0.6146	0.9989	0.9592
BPZ	0.492–151.359	−0.0013	0.5379	0.9996	0.3345
BPAF	0.510–157.160	0.0018	1.0278	0.9992	0.1047
TBBPA	0.497–152.889	0.0052	1.0845	0.9991	0.2226
BPP	0.496–152.719	−0.0019	0.6257	0.9992	0.2031

times of the corresponding bisphenols. No interferents were observed at the retention times of analytes ±0.1 min in any of the transitions.

3.4.3. Matrix effects

Suppression or enhancement of the analyte signal is a common phenomenon in ESI and should be properly evaluated during method validation. Thus, matrix effects were assessed by the post-extraction addition method, which is based on the comparison of the responses obtained for a spiked extract with those obtained for a standard solution at the same concentration. The percent matrix effect (%ME) was calculated as $(R_{se}/R_{std} - 1) \times 100$, where R_{se} is the response of the analyte in the spiked extract and R_{std} is the corresponding response in the standard solution [39,40]. In this context, a negative result indicates ionization suppression, whereas a positive result indicates signal enhancement. These experiments were performed using a composite meal extract spiked at three concentration levels equivalent to 1.0, 15.0 and 30.0 $\mu g\,kg^{-1}$. As shown in Table 4, matrix effects ranged from 26% for 2,2′-BPF at the lowest concentration level to about −24% for BPAP at 30 $\mu g\,kg^{-1}$. Among the studied compounds, five out of thirteen (BPE, BPA, BPZ, TBBPA and BPP) presented matrix effects within ±10%, and only two compounds (2,2′-BPF and BPAP) showed absolute matrix effects higher than 20%. Although the observed %MEs were significant for most bisphenols, they can still be considered satisfactory, especially when considering the complex nature of the analyzed samples.

3.4.4. Repeatability and intermediate precision

The precision of the method was evaluated both at repeatability and intermediate precision conditions, using a composite meal sample spiked at 1.0, 15.0 and 30.0 $\mu g\,kg^{-1}$. Three subsamples of each concentration level were analyzed under repeatability conditions (same operator, same laboratory and same equipment) on five days. Homogeneity of variances was checked by Cochran test and then analysis of variance (ANOVA) was applied to estimate within-days variance (σ^2_{within}) and between-days variance ($\sigma^2_{between}$). Repeatability was expressed as percent relative standard deviation (%RSD_r) calculated by dividing the root square of σ^2_{within} by the overall mean of the determinations. Intermediate precision (%RSD_{IP}) calculated by dividing the root square of the total variance ($\sigma^2_{within} + \sigma^2_{between}$) by the overall mean of the determinations (Table 4). Both, repeatability and intermediate precision were satisfactory, showing RSDs values ≤7.8 % and ≤10 %, respectively.

3.4.5. Measurement uncertainty

Evaluation of measurement uncertainty was carried out by the combination of the bottom-up approach and the in-house validation data as suggested by the Eurachem/CITAC guide [41]. Main sources of uncertainty, including standards and sample preparation, intermediate precision, calibration and bias, were quantified and combined standard uncertainties (u_c) were calculated according to the law of error propagation. The expanded uncertainties (U) were finally estimated using a coverage factor (k) of 2, corresponding to a confidence level of 95% (Table 4). As shown, the relative expanded uncertainties ranged, depending on the analyte, from 8.5% for BPAF to 16.6% for BPA.

3.4.6. Trueness

Due to the lack of certified reference materials (CRM) for bisphenols in food, recovery experiments were performed for assessing the trueness of the method. A composite meal sample spiked at 1.0, 15.0 and 30.0 $\mu g\,kg^{-1}$ was used for the recovery experiments. Samples were analyzed in triplicate over five days and bias was estimated for each analyte as the difference between the measured and the added concentration (Table 5). The magnitude of bias was expressed in terms of zeta-scores, which evaluate the agreement of the measured value with the nominal value, considering measurement uncertainty [42]. Bias was statistically not significant for any of the analyte/concentration level combinations, as all zeta-scores were well below the absolute level of two (95% confidence interval).

3.4.7. Limits of detection

Since blank correction was applied for the quantification of BPA, it was also used for the estimation of the LODs as suggested by the Eurachem guide on analytical methods validation [38]. A composite meal sample spiked at 1.0 $\mu g\,kg^{-1}$ was analyzed ($n = 10$) under repeatability conditions and the SD was calculated after procedural blank correction ($n = 10$). The SD was then corrected according to Eq. (1) as follows:

$$SD_c = SD\sqrt{\frac{1}{n} + \frac{1}{n_b}} \quad (1)$$

where n denotes the number of sample replicates and n_b is the number of procedural blank replicates used for the blank correction. The LOD and the limit of quantification (LOQ) were then estimated as three and ten times the SD_c, respectively (Table 5). A LOD of 0.073 $\mu g\,kg^{-1}$ was obtained for BPA following this approach.

For the rest of the bisphenols, LODs were estimated from a composite meal sample spiked at low decreasing concentration levels. LODs were calculated as the average concentrations of compound producing a signal-to-noise ratio (S/N) of 3 using the less sensitive MS/MS transition (MRM2), i.e. the one permitting the unambiguous identification of the analytes. On the other hand, LOQs were estimated as the average concentrations of compound producing a S/N of 10 using the most sensitive MS/MS transition (MRM1), provided that the S/N for MRM2 was at least of 3. The proposed method provided low LODs, which ranged from 0.025 $\mu g\,kg^{-1}$ for BPAF to

Table 4
Matrix effects, repeatability, intermediate precision and relative expanded uncertainty.

Compound	% ME ± SD ($n = 3$)			RSD_r (%) (1.0 μg kg^{-1})	RSD_{IP} (%) (1.0 μg kg^{-1})	RSD_r (%) (15.0 μg kg^{-1})	RSD_{IP} (%) (15.0 μg kg^{-1})	RSD_r (%) (30.0 μg kg^{-1})	RSD_{IP} (%) (30.0 μg kg^{-1})	U (%, $k = 2$)
	1.0 μg kg^{-1}	15.0 μg kg^{-1}	30.0 μg kg^{-1}							
BPS	−11.3 ± 3.2	−16.6 ± 3.6	−18.0 ± 4.7	5.4	5.4	1.9	2.3	2.2	2.6	10.0
DMBPS	−5.6 ± 7.4	−9.6 ± 4.4	−14.7 ± 5.7	5.9	6.7	5.7	6.2	5.7	6.4	13.2
2,2′-BPF	26.0 ± 8.8	10.9 ± 4.2	2.6 ± 5.6	4.5	6.6	4.3	4.3	3.5	4.2	12.3
2,4′-BPF	−9.1 ± 6.3	−16.7 ± 3.5	−20.2 ± 5.1	5.7	5.7	4.2	7.0	4.7	4.8	13.5
4,4′-BPF	−3.5 ± 6.3	−13.9 ± 3.6	−15.9 ± 5.3	5.1	5.9	2.1	2.1	3.3	3.4	13.1
BPE	0.47 ± 8.1	-5.1 ± 4.3	−8.7 ± 5.1	4.7	4.7	1.8	2.0	1.2	1.3	16.6
BPA	9.3 ± 10.2	1.1 ± 4.4	−6.1 ± 4.7	4.9	4.9	2.2	2.4	1.6	1.9	16.6
BPB	2.5 ± 9.9	−5.7 ± 3.7	−12.2 ± 3.9	5.2	5.2	2.4	2.4	1.9	2.0	12.4
BPAP	−20.2 ± 6.7	−21.0 ± 3.3	−24.3 ± 4.6	7.7	7.7	2.4	2.8	2.9	5.2	12.8
BPZ	8.1 ± 9.2	−2.8 ± 6.1	−7.0 ± 5.8	7.8	10.0	2.5	5.2	4.0	4.7	15.9
BPAF	−7.2 ± 7.2	−11.9 ± 2.4	−12.8 ± 5.0	3.4	3.4	2.2	2.2	1.6	1.7	8.5
TBBPA	4.1 ± 6.5	−2.2 ± 1.5	−6.7 ± 4.4	4.5	5.9	2.1	2.4	2.7	3.5	11.6
BPP	6.6 ± 8.6	1.4 ± 3.1	−3.1 ± 6.0	6.5	7.0	3.4	3.4	4.2	4.9	12.8

Table 5
Trueness assessment and limits of detection and quantification of the proposed method.

Compound	Bias ± SD ($n = 5$, μg kg^{-1}) (1.0 μg kg^{-1})	z-score (1.0 μg kg^{-1})	Bias ± SD ($n = 5$, μg kg^{-1}) (15.0 μg kg^{-1})	z-score (15.0 μg kg^{-1})	Bias ± SD ($n = 5$, μg kg^{-1}) (30.0 μg kg^{-1})	z-score (30.0 μg kg^{-1})	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
BPS	0.025 ± 0.022	0.26	0.381 ± 0.208	0.25	0.642 ± 0.580	0.21	0.140	0.334
DMBPS	0.033 ± 0.035	0.27	0.440 ± 0.377	0.22	1.281 ± 1.075	0.30	0.037	0.083
2,2′-BPF	0.039 ± 0.025	0.35	0.227 ± 0.170	0.13	0.530 ± 0.661	0.15	0.043	0.092
2,4′-BPF	0.012 ± 0.010	0.10	0.630 ± 0.436	0.30	0.694 ± 0.625	0.16	0.110	0.199
4,4′-BPF	0.042 ± 0.027	0.33	0.581 ± 0.166	0.28	1.506 ± 0.670	0.36	0.082	0.193
BPE	0.035 ± 0.018	0.22	1.200 ± 0.229	0.44	2.878 ± 0.389	0.52	0.031	0.053
BPA	0.033 ± 0.016	0.18	1.530 ± 0.296	0.49	3.077 ± 0.527	0.50	0.073	0.243
BPB	0.049 ± 0.023	0.44	0.688 ± 0.053	0.37	1.263 ± 0.382	0.34	0.033	0.081
BPAP	0.016 ± 0.011	0.13	0.238 ± 0.136	0.11	1.098 ± 0.719	0.26	0.031	0.039
BPZ	0.052 ± 0.035	0.33	0.612 ± 0.392	0.24	0.992 ± 0.482	0.19	0.038	0.038
BPAF	0.018 ± 0.013	0.20	0.363 ± 0.146	0.25	0.732 ± 0.299	0.25	0.025	0.066
TBBPA	0.034 ± 0.026	0.29	0.319 ± 0.274	0.17	0.697 ± 0.741	0.18	0.059	0.178
BPP	0.034 ± 0.020	0.26	0.117 ± 0.128	0.06	0.766 ± 0.546	0.18	0.031	0.072

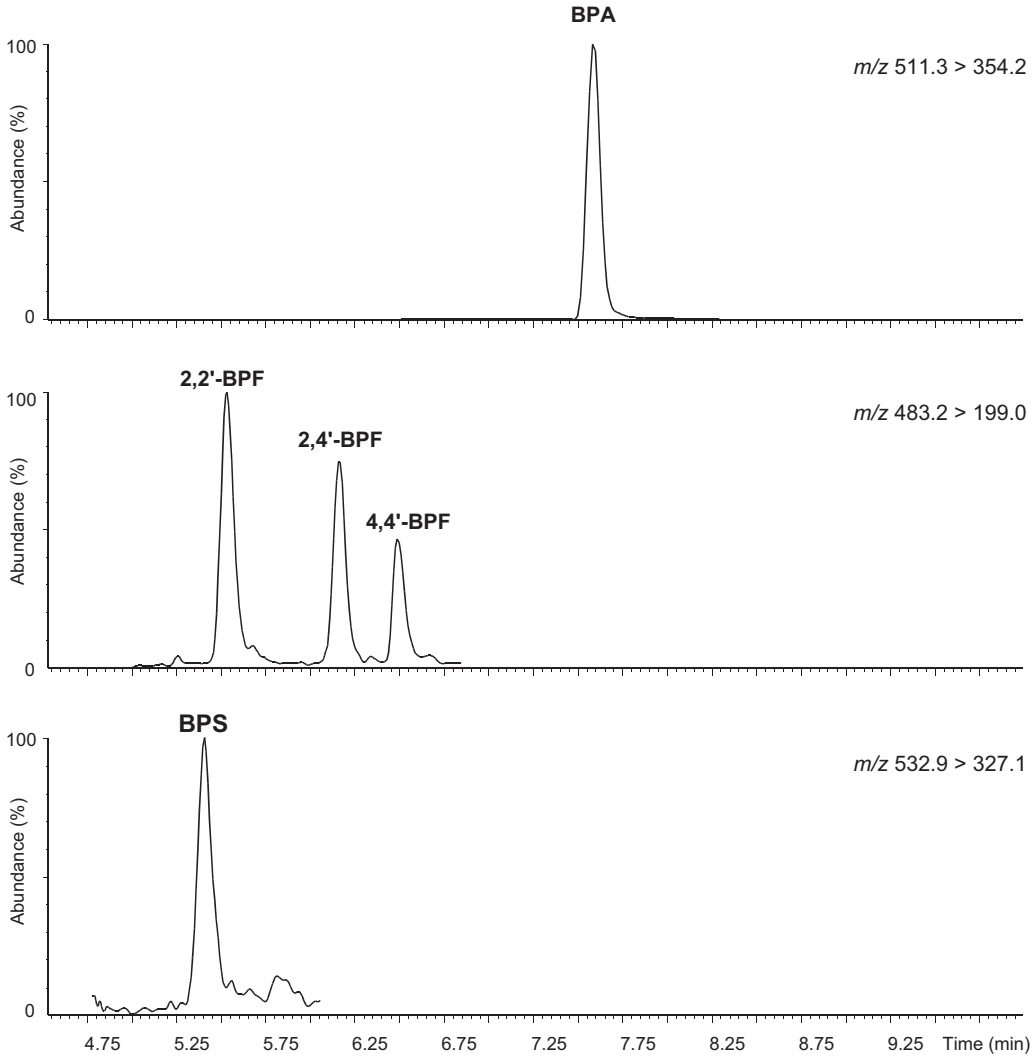


Fig. 6. MRM chromatograms obtained for canned beef ravioli.

0.140 μg kg^{-1} for BPS. These values are between 10- and 20-fold lower than those reported for BPA analogs by Alabi et al. [14] and Cunha et al. [10,11], and similar to those obtained by Yang et al. [13] in canned foods.

3.5. Application to real ready-made meals

Eventually, the proposed method was applied to several ready-made meal samples from Belgium. The analyzed samples included the following dishes: pork filet with mixed vegetables, veal cutlet Milanese with pasta, fried sausage with mixed legumes, ham salad, spaghetti Bolognese, sweet and sour chicken, tuna salad, beef ravioli, and ham and mushroom pizza. Three procedural blanks were analyzed in the same sequence and the average value for BPA (0.062 ± 0.002 μg kg^{-1}) was subtracted. BPA was detected in all the analyzed samples at concentrations ranging from <LOQ to 17.7 ± 2.9 μg kg^{-1} for canned beef ravioli. No bisphenol analogs were detected in any of the samples, with the exception of BPS and the BPF isomers, which were present in the beef ravioli. BPS was found in this sample at 2.4 ± 0.2 μg kg^{-1}, whereas the BPF isomers ranged from 0.37 ± 0.05 μg kg^{-1} for 4,4′-BPF to 0.39 ± 0.05 μg kg^{-1} for 2,4′-BPF. Fig. 6 shows the MRM chromatograms obtained for this sample.

4. Conclusions

In this work, a simple and robust method based on SID-LC–MS/MS for the determination of BPA and 12 other bisphenol analogs (BPS, DMBPS, 2,2′-BPF, 2,4′-BPF, 4,4′-BPF, BPE, BPB, BPAP, BPZ, BPAF, TBBPA and BPP) in ready-made meals is presented. Due to the enormous variety of ready-made meals available on the market, the proposed method has been designed to cover a broad range of solid foodstuffs. Therefore, its suitability not only for ready-made meals but also for the analysis of bisphenols in other complex solid food samples is of advantage.

Ultrasound assisted extraction with acetonitrile/methanol (80:20, v/v) preceded by sample disruption with sand was demonstrated to be an inexpensive and efficient procedure for the extraction of bisphenols from complex foodstuffs. In addition, the use of fresh samples instead of freeze-dried samples constitutes a substantial saving in total analysis time, which allows increasing sample throughput. A selective clean-up of sample extracts was achieved by using SPE with PSA sorbent.

ESI ionization efficiency of bisphenols was highly improved by applying a simple derivatization step with PS chloride, which allowed decreasing the LODs of the method to levels ranging from 0.025 μg kg^{-1} for BPAF to 0.140 μg kg^{-1} for BPS. The unique selectivity of a pentafluorophenylpropyl HPLC stationary phase

provided sufficient resolution for all studied bisphenol derivatives. Baseline resolution was achieved for the three BPF isomers, which made individual quantification possible. Performance of the method was evaluated in terms of selectivity, matrix effects, linearity, precision, measurement uncertainty, trueness, LODs and LOQs. Repeatability and intermediate precision were satisfactory, showing RSDs values ≤7.8% and ≤10%, respectively. The estimated relative expanded uncertainty ($k = 2$) was below 17% in all cases, and bias was not significant for any of the bisphenols. In summary, the proposed method complies with performance criteria set in European legislation for other food contaminants such as polycyclic aromatic hydrocarbons.

The applicability of the proposed method was assessed by analyzing several popular ready-made meals purchased from supermarkets in Belgium. BPA was detected in all analyzed samples up to $17.7 \pm 2.9\ \mu g\ kg^{-1}$. Among the BPA analogs, only BPS, 2,2′-BPF, 2,4′-BPF and 4,4′-BPF were found in canned beef ravioli.

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